

# **Antigen D Analysis Workflow**

Daniela Borgmann, April 2015







# **Antigen D Analysis Workflow**





D. Borgmann, Antigen D Analysis Workflow

# **Start Antigen D Analyse**



(🏲 NanoDetec	t v. 1.0	
File User	Analysis Arrange Help	
	Structure Detection	
	Spot Detection	Single Molecule Analysis
		Trajectory Analysis
		Cluster Analysis
		Antigen D Analysis
		Antigen D Result Merger

## Antigen D Analysis

Start analysis of newly measured antigen D data: cell detection, molecule detection, statistics, export functionality

## **Antigen D Result Merger**

Start result merger: analysed and exported data files can be imported and visualized; machine learning can be started





# **Import Antigen D Analyse**



## **Add New Group**

A new group can be added with specific name and specific color (used for result visualization)

# Add Group Dialog New Group Name: OK



### Add new Images

New images can be added using the fileimporter (see next





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# Import SPE Images 1



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# **Import SPE Images 2**







#### **Import Options:**

- <u>Begin Fluro</u> = First Frame to be analysed
- <u>End Fluro</u> = Last Frame to be analysed
- <u>White light</u> = Frame Number White light image
- Add to Group = Select the group samples shall belong to
- <u>Scale Factor</u> = Select scaling factor of images (preview can be seen on the right handside)



# **Start Automatic Analysis**



#### Start Analysis:

Antigen D analysis is performed automatically for all selected files; running files are marked as following:



Workflow

# Show and Save Results Automatic Analysis



NanoDetect	. v. 1.0 (Admin)		and instantion	a thread hand has								
File User	Analysis Arrange Help											
🕐 Antigen D	Analysis										3	
File Sett	ings											
🖃 🐨 🖓 🗛	igen D Analysis	Included Results	Intensity Results Ce	I Intensities Distribution Res	sults Distribution Cell Inter	nsities Cell Compa	arisons Data Summary				_	
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	Dweak 2015022 wd3_H41_1_20_top_area12_rot_013_SPE 20150224_rd3_H41_1_20_top_area13_rot_014_SPE	Z0150224_wd3_H41_1_20_top_area3_rot_002_SPE     Z0150407_DH41_1_20_top_area4_rot_036_SPE     Z0150407_DH41_1_20_top_area5_rot_036_SPE     Z0150407_D_H41_1_20_top_area5_rot_038_SPE		Dwea	k			D+		D-		
÷	20150224_wdt H41_1_20_top_area 15_rot_017SPE 20150224_wd3_V41_1_20_top_area 22_rot_024SPE 20150224_wd3_H4_1_20_top_area 3_rot_002SPE		1073					— <u> </u>				
	20150407_DH41_1_20_top_area4_rot_036_SPE	20150407_D+_H41_1_20_top_area5_rot_009SPE     20150407_D+_H41_1_20_top_area6_rot_010SPE     20150407_D+_H41_1_20_top_area8_rot_013_SPE     20150407_D+_H41_1_20_top_area8_rot_013_SPE	780	-				_				
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Options		Prished Importing	-100	Intensity Results Cell Int Image Result Frame R Group	ensities Distribution Research	sults Distribution	Cell Intensities Cell I Average Intensity	Comparisons Data : Median	Summary	3rd Quantil	Distance All Average	Dista Near Avera
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Options	Antigen D Analys	Frished Importing	-100	Intensity Results Cell Int Image Result Frame R Group Dweak Dweak Dweak Dweak	Instribution Research           Filename           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3	Single           Single           Molecules           1273           1517           805           1014           698	Average Intensities         Cell Intensities           868.895         948.977           906.865         930.284           899.536	Median           871.243           949.996           912.115           931.73           905.828	Summary   1st Quantil 862,957 948,761 893,396 921,921 884,986	3rd Quantil           868,895           948,977           906,865           930,284           899,536	Distance All Average 24.245 25.498 24.47 26.149 22.547	Dista Near Avera           4.684           4.072           4.143           3.217           4.392
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Options	Antigen D Analys After clicking on "Antiger results are combined an	sis: en D Analysis" all calcu d visualized in serveral v	lated vays.	Intensity Results Cell Int Image Result Frame R Group Desk Dweak Dweak Dweak D- D- D- D- D- D-	Distribution Research           Distribution Research           Pilename           20150224_wd3           20150207_D_H           20150407_D_H           20150407_D_H           20150407_D_H           20150407_D_H           20150407_D_H	Single         Count           1273         1517           1517         1014           698         1           0         0           0         640	Cell Intensities Cell I	Median           871,243           949,996           912,115           931,73           905,828           0           0           0           0           0           0	Summary ] 1st Quantil 862,957 948,761 893,396 921,921 884,986 0 0 0 0 0 0 0 0 0 0 0 0 0	3rd Quantil           868.895         948.977           906.865         930.284           899.536         0           0         0           0         0           0         0           0         0	Distance All Average           24,245           25,498           24,47           26,149           22,547           n. def.           n. def.           n. def.           n. def.           n. def.	Dista Near Avera           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           0           0           0
Options	Antigen D Analys After clicking on "Antiger results are combined an Using the Data Suma	sis: an D Analysis" all calcu d visualized in serveral v mry tab, all results cal	lated vays. n be	Intensity Results Cell Int Image Result Frame R Group Dweak Dweak Dweak Dweak D- D- D- D- D- D- D-	Instruction         Distribution         Research           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150207_D-JH         20150407_D-JH         20150407_D-JH           20150407_D-JH         20150407_D-JH         20150407_D-JH	Log Count Single Molecules     Log Count Single Molecules     Log Count Single Molecules     Log Count 1273     Log Count 1517     L	Cell Intensities         Cell Intensities           Recarge Intensity         Average Intensity           868.957         906.865           930.284         899.536           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0	Data           871,243           949,996           912,115           931,73           905,828           0           0           0           0           0           0           0           0           0           0           0           0           0	Summary ] Ist Quantil 862,957 948,761 893,396 921,921 884,586 0 0 0 0 0 0 0 0 0 0 0 0 0	3rd Quantil           868,895           948,977           906,865           930,284           899,536           0           0           0           0           0           0           0           0           0           0           0	Distance All Average           24.245           25.498           24.47           26.149           22.547           n. def.           n. def.           n. def.           n. def.           n. def.           25.922	Dista Near Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           5.453           5.172
Options	After clicking on "Antiger results are combined an Using the Data Sumar	sis: en D Analysis" all calcu d visualized in serveral v mry tab, all results cal	lated vays. n be	Intensity Results Cell Int Image Result Frame R Broup Dweak Dweak Dweak Dweak Dweak Dweak D- D- D- D- D- D- D- D- D-	Instruction         Distribution         Research           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         2015024_wd3         2015024_wd3           2015024_wd3         20150407_D_H         20150407_D_H           20150407_D_H         20150407_D_H         20150407_D_H           20150407_D_H         20150407_D_H         20150407_D_H           20150407_D_T_H         20150407_D_T_H         20150407_D_T_H	Distribution           Count Single Molecules           1273           1517           805           1014           698           1           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0	Cell Intensities         Cell Intensities           Reference         Average           Intensity         968.895           948.977         906.865           930.284         899.536           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0	Data           871.243           949.396           912.115           931.73           905.828           0           0           0           799.327           840.743	Summary Ist Quantil 862.957 948.761 893.396 921.921 884.986 0 0 0 0 0 0 0 0 784.729 827.509 827.509 829.6	3rd Quantil           868.895           948.977           966.865           930.284           899.536           0           0           0           0           0           795.361           837.025           920.201	Distance All Average           24.245           25.498           24.47           26.149           22.547           n. def.           n. def.           n. def.           n. def.           z2.922           25.624	Dista Near Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0      0           0      0
Options	Antigen D Analys After clicking on "Antige results are combined an Using the Data Sumar exported as *.csv using	<b>SiS:</b> en D Analysis" all calcu d visualized in serveral v mry tab, all results can the export button (those	lated vays. n be files	Intensity Results Cell Int Image Result Frame R Group Dweak Dweak Dweak Dweak D- D- D- D- D- D- D- D- D- D-	Distribution Research           Distribution Research           Pilename           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150407_DH	Distribution           Count Single Molecules           1273           1517           805           1014           698           1           0           0           0           0           1026           601           1326	Cell Intensities         Cell Intensities           Research         Average           948.957         948.977           906.865         930.284           899.536         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0	Data           871,243           949,396           912,115           931,73           905,828           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0           0 <td>Summary         Ist Quantil           862.957         948.761           933.396         921.921           884.986         0           0         0           0         0           0         0           0         20           0         20           0         20           0         20           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0</td> <td>3rd Quantil           868.895           948.977           906.865           930.284           899.536           0           0           0           795.361           837.025           830.799</td> <td>Distance All Average           24.245           25.498           24.7           26.149           22.547           n. def.           n. def.           n. def.           22.922           25.624           25.621</td> <td>Dista Near Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           0           5.453           5.137           5.582</td>	Summary         Ist Quantil           862.957         948.761           933.396         921.921           884.986         0           0         0           0         0           0         0           0         20           0         20           0         20           0         20           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0	3rd Quantil           868.895           948.977           906.865           930.284           899.536           0           0           0           795.361           837.025           830.799	Distance All Average           24.245           25.498           24.7           26.149           22.547           n. def.           n. def.           n. def.           22.922           25.624           25.621	Dista Near Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           0           5.453           5.137           5.582
Options	Antigen D Analys After clicking on "Antige results are combined an Using the Data Suma exported as *.csv using can be imported using th	en D Analysis" all calcu d visualized in serveral v mry tab, all results can the export button (those ne Result Merger)	lated vays. n be files	Intensity Results Cell Int Image Result Frame R Dweak Dweak Dweak Dweak Dweak Dweak Dweak Dweak Dweak Dweak D- D- D- D- D- D- D- D- D- D- D- D- D-	Distribution Research           Filename           20150224_wd3           2015024_wd3           20150407_D-,H	Uts         Distribution           Count Molecules         Single Molecules           1273         1517           805         1014           698         1           0         0           0         649           1026         601           1335         1	Cell Intensities Cell I	Median           871.243           949.996           912.115           931.73           905.828           0           0           0           949.9327           840,743           839.843           861.706	Summary Ist Quantil 862,957 948,761 893,396 921,921 884,986 0 0 0 0 0 0 0 0 0 0 0 0 0	3rd Quantil           868,895           948,977           906,865           930,284           899,536           0           0           0           0           0           0           943,977           906,865           930,284           899,536           0           0           0           0           0           0           0           0           0           0           0           0           0           0           930,281           830,799           858,811	Distance All Average           24,245           25,498           24,47           26,149           22,547           n. def.           n. def.           z2,562           25,692           25,624           25,701	Dista Near Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           0           0           0           5.453           5.137           5.582           4.106
	Antigen D Analys After clicking on "Antiger results are combined an Using the Data Sumar exported as *.csv using can be imported using the	en D Analysis" all calcu d visualized in serveral w mry tab, all results can the export button (those ne Result Merger)	lated vays. n be files	Intensity Results Cell Int Image Result Frame R Group Desk Dweak Dweak Dweak Desk D- D- D- D- D- D+ D+ D+ D+	Distribution Resease           Distribution Resease           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150224_wd3           20150274_wd3           20150407_DH	Uter bit in the second	Cell Intensities         Cell I           Recently         Recently           968,895         948,957           906,865         930,284           899,536         0           0         0           0         330,284           893,536         330,284           893,536         330,284           906,865         330,284           893,536         330,284           906,865         330,284           893,636         330,284           893,636         330,284           833,025         833,739           858,811         858,811	Comparisons Data 871,243 949,996 912,115 931,73 905,828 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Summary Ist Quantil 862,957 948,761 893,396 921,921 884,986 0 0 0 0 0 0 0 784,729 827,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,509 822,51 822,51 822,51 823,51 823,51 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 824,751 825,752 825,752 825,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,752 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,753 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 827,755 8	3rd Quartil           868.895           948.977           906.865           930.284           899.536           0           0           0           0           839.536           93.284           89.536           93.284           89.536           93.284           89.536           93.284           89.536           93.284           830.795           830.799           858.811	Distance All Average           24,245           25,498           24,47           26,149           22,547           n. def.           n. def.           n. def.           25,592           25,642           25,701	Dista           Nea           Aver           4.684           4.072           4.143           3.217           4.392           0           0           0           0           5.453           5.137           5.582           4.106
Options Control Control Contr	After clicking on "Antiger results are combined an Using the Data Sumar exported as *.csv using can be imported using the D. Borgmann, Antigen D	en D Analysis" all calcu d visualized in serveral v mry tab, all results can the export button (those ne Result Merger)	lated vays. n be files	Intensity Results Cell Int Image Result Frame R Group Dweak Dweak Dweak Dweak D- D- D- D- D- D+ D+ D+ D+	Instruction         Distribution         Research           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150224_wd3         20150224_wd3           20150224_wd3         20150274_wd3         20150407_DH           20150407_DH         20150407_DH         20150407_DH           20150407_DH         20150407_D+_H         20150407_D+_H	Letropolition     Logithered	Cell Intensities         Cell I           868,855         948,957           906,865         930,284           899,536         0           0         0           0         330,295           837,025         833,799           858,811         358,811	Comparisons Data           Data           871,243           949,996           912,115           931,73           908,828           0           0           0           90           799,327           840,743           838,843           861,706	Summary           Ist Quantil           862.957           948.761           893.396           921.921           884.985           0           0           0           0           0           827.509           822.6           851.739	3rd Quantil           868,895           948,977           906,865           930,284           899,536           0           0           0           0           830,795           858,811	Distance All Average           24,245           25,498           24,47           26,149           22,547           n. def.           n. def.           n. def.           22,922           25,402           25,701	Dista           Nea           Aver           4.584           4.072           4.143           3.217           4.392           0           0           0           0           5.552           4.106



# **Start Manual Analysis**



#### Manual Antigen D Analysis:

Each image can be analysed manually by clicking one the image name on the left handside. The different analysis steps include cell detection, molecule detection, and result calculation.



# Manual Analysis Cell Detection







# Manual Analysis Molecule Detection









# **Antigen D Result Merger**





# **Antigen D Result Merger**



Features can be selected and be plotted; average values are calculated for each group independently

D. Borgmann, Antigen D Analysis Workflow

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